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Research paper

A model for the dynamics of the parasitic stages of equine cyathostomins

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ABSTRACT

A model was developed to reproduce the dynamics of the parasitic stages of equine cyathostomins. Based on a detailed review of published literature, a deterministic simulation model was constructed using the escalator boxcar-train approach, which allows for fully-overlapping cohorts of worms and approximately normally distributed variations in age/size classes. Key biological features include a declining establishment of ingested infective stage larvae as horses age. Development rates are constant for all the parasitic stages except the encysted early third stage larvae, for which development rates are variable to reflect the sometimes extended arrestment of this stage. For these, development is slowed in the presence of adult worms in the intestinal lumen, and when ingestion of infective larvae on herbage is high or extended. In the absence of anthelmintic treatments, the life span of adult worms is approximately 12 months, and the presence of an established adult worm burden largely blocks the transition of luminal fourth stage larvae to the adult stage, resulting in mortality of the larvae. This inhibition is removed by effective anthelmintic treatment allowing the rapid replacement of adult worms from the pool of mucosal stages.

Within the model, the rate and seasonality at which infective stage larvae are ingested strongly influences the dynamics of the pre-adult stages. While the adult worm burden remains relatively stable within a year, due to the negative feedback they have on developing stages, the numbers and proportions of larval stages relative to the total worm burden increase with the numbers of infective larvae ingested. Further, within the model, the seasonal rise and fall of encysted stages is largely driven by the seasonal pattern of infective larvae on pasture. Because of this, the model reproduces the contrasting seasonal patterns of mucosal larvae, typical of temperate and tropical environments, using only the appropriate seasonality of larvae on pasture. Thus, the model reproduces output typical of different climatic regions and suggests that observed patterns of arrested development may simply reflect the numbers and seasonality of free-living stages on pasture as determined by different management practices and weather patterns.

1. Introduction

Nematode parasites of the subfamily Cyathostominae constitute a complex of 50 species, commonly referred to as cyathostomins or small strongyles (Lichtenfels et al., 2008). These parasites are extremely widespread, with infections being ubiquitous in grazing horses around the world (Lyons et al., 1999). Since the advent and widespread use of modern anthelmintics, and the consequent decline in prevalence of the highly pathogenic large strongyle *Strongylus vulgaris*, the cyathostomins have come to be regarded as the most important strongylid parasites of the horse (Love et al., 1999; Lyons et al., 2011).

The widespread development of resistance to anthelmintics by the cyathostomins is therefore a concern as it could ultimately threaten the utility of these drugs for controlling cyathostomin infections (Kaplan, 2004; Peregrine et al., 2014). Resistance to two anthelmintic classes, the benzimidazoles and tetrahydropyrimidines, is now common and reported worldwide (Kaplan, 2004; Peregrine et al., 2014; Matthews, 2004a,b), while recent evidence suggests emerging resistance to the third class, the macrocyclic lactones (Peregrine et al., 2014; Bellaw et al., 2018). In response, efforts have been made to develop and promote strategies intended to slow further development of resistance, such as targeting anthelmintic treatments to horses on the basis of faecal egg counts (Gomez and Georgi, 1991; Duncan and Love, 1991). However, the validity of these approaches remains unconfirmed (Nielsen et al., 2014), at least in part due to the lack of detailed understanding of the biology of cyathostomin parasites (Herd and

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Willardson, 1985) and how to manage both parasitism and drug use in order to minimise selection for resistance (Reinemeyer, 1999; Nielsen et al., 2014).

The development and analysis of models has been a useful adjunct to research programmes investigating the prevention and management of resistance in nematode parasites of sheep (Smith, 1990; Barnes et al., 1995; Learmount et al., 2010) and in other disease organisms (Hastings, 2001). It is highly likely that they would prove equally useful in understanding and managing resistance development in parasites of horses (Leathwick et al., 2016, 2017). While the basic life cycle of the cyathostomins is well known, the variables determining population size and dynamics of the various life cycle stages are poorly understood (Ogbourne, 1975; Nielsen et al., 2014). Herein, we describe a model for the dynamics of cyathostomin infection in the horse, focusing on the progression of parasitic stages.

2. Materials and methods

2.1. Parasite biology

While cyathostomin infections invariably consist of a complex of genera and species, the same five to 10 tend to predominate in prevalence and intensity throughout the world (Anderson and Hasslinger, 1982; Reinemeyer et al., 1984; Krecek et al., 1989; Lind et al., 2003; Kuzmina et al., 2005). Some of these species show subtle differences in biology (Chapman et al., 2003; Lyons et al., 2011; Nielsen et al., 2014), but in general the cyathostomins are regarded as a single biological entity. Given the limited knowledge available regarding the variables driving worm dynamics on a species-specific level within the host, a single model representing the cyathostomin species complex is the only practical option at this time (Nielsen et al., 2014).

The development of cyathostomins within the host is straightforward. After ingestion, the infective third stage larva (L3) exsheaths and migrates into the tubular glands of the large intestinal mucosa or submucosa where it becomes encapsulated by a host tissue reaction. After a variable period, the early L3 (EL3) develops into a late L3 (LL3), which subsequently moults to a mucosal L4. The L4 excysts, emerges from the mucosa, and migrates into the gut lumen where the final moult to adult occurs (Ogbourne, 1975). A notable characteristic of the cyathostomins is that EL3s arrest their development for what can be a protracted period (Gibson, 1953; Smith, 1976). While this basic life-history is obviously rigid, the numbers in each stage can vary enormously, even between animals of similar age and experience (Nielsen et al., 2010).

2.2. Model structure and driving variables

The model was structured to reflect the various stages of the parasite life cycle, *i.e.*, L3 (the infective stage ingested with herbage), EL3 within the mucosa (the stage that arrests), LL3/L4 (collectively termed 'developing larvae') within the mucosa, and L4 and adults in the gut lumen (Fig. 1). As with previous models (Leathwick et al., 2015, 2016), the different stages were modelled using the escalator boxcar train approach (De Roos, 1988; De Roos et al., 1992) using Microsoft Excel software. This approach uses an array to store the number of individuals in a series of age or size classes as they transition from cell to cell at predetermined or variable rates. A major advantage of this approach, apart from its' simplicity, is that it allows for fully overlapping cohorts of individuals and results in an approximately normal distribution of development times or growth rates. In this case, the same modelling approach allowed for differing development rates of EL3 (consistent with variable durations of arrested development) and different age classes of adult worms (so that worms surviving anthelmintic treatment were older, with shorter life expectancies, than those that replaced them from the pool of mucosal larvae).

2.2.1. Establishment of ingested larvae

Horses acquire some immunity against cyathostomin infection with age and experience of infection, although this tends to be slow to develop and is generally incomplete in that most horses harbour significant populations of these nematodes (Klei and Chapman, 1999). Total worm numbers are typically higher in immature than mature animals (Love and Duncan, 1992; Bucknell et al., 1994) and yearlings tend to have a lower proportion of EL3 and a higher abundance of adult worms than older horses (Reinemeyer et al., 1988; Chapman et al., 2003). However, this pattern appears to be highly variable (Nielsen et al., 2010).

A small number of studies have involved infection of horses with known numbers of larvae followed by necropsy for worm count, so these studies were useful for estimating the percentage of L3 establishing. Data in Murphy and Love (1997) showed a sizable variation in establishment of challenge infection between individual horses even though the subjects were all young animals (6–12 months old). Consequently, because mean values from different studies would serve as the basis for estimates of establishment rates in relation to horse age, only those studies with group sizes of ≥3 were considered. Data presented in Reinemeyer et al. (1988), Murphy and Love (1997), Monahan et al. (1997, 1998), Chapman et al. (2002) and Baudena (2003) were used to estimate the proportion of each challenge infection, which successfully established in horses of different ages. A linear function was fitted to the proportion of the challenge dose recovered at necropsy (the sum of all life-history stages), after transformation by Log_e, against horse age, to yield the following equation;

$$Est_{L3} = e^{-1.919-0.118\,'horse\ age'} \quad (1)$$

Where Est_{L3} = the proportion of ingested L3 establishing, and horse age = the age of the horse in years. This returns an establishment rate, which declines, exponentially from 14.7% in foals in their first year of life to 1.4% in horses of 20 years.

2.2.2. The duration of parasitic stages

Development of the EL3 stage from ingestion to developing larvae (*i.e.*, LL3-L4) was modelled using an array with 10 cells. Individuals progressed from one cell to the next at a rate (R_{EL3}) determined by;

$$R_{EL3} = e^{-\alpha.A.B} \quad (2)$$

Where $\alpha = 0.0001$, A = (horse age) $^{0.333}$ and B = ($L3_i$ + WB)

Where 'horse age' = the age of the horse in years, $L3i$ = the average daily intake of L3 over the previous 6 weeks and WB = the current adult worm burden. This relationship modifies the rate of development of EL3 in the short-term (*i.e.,* within a year) based on the presence of an established adult worm burden and the rate at which L3 are ingested with herbage. Thus, development rate is reduced by negative feedback from the presence of adult nematodes in the lumen and large larval challenges or 'trickle' infections (Murphy and Love, 1997; Baudena, 2003). In addition, Eq. (2) reduces the development rate in the longer term (*i.e.,* over years) with the increasing age of the horse. This reflects the development of immunity as horses age, and the associated slower development and subsequent increase in the proportion of EL3 seen in worm counts from older horses (Chapman et al., 2003). No experimental data existed to fit the coefficients for this relationship, *i.e.,* while both ingestion of L3 and the presence of adult worms are known to influence the duration of the EL3 stage, their relative importance is not known. Therefore, coefficients were chosen to yield realistic times for the duration of development, based on published literature (Round, 1969; Reinemeyer et al., 1988; Lyons et al., 2011).

The developing LL3 and L4 stages were represented in the model by six cells and a constant rate of development, which results in a median development time of 43 days with 90% of individuals completing development between 20 and 75 days. Adult worms were represented by 30 cells (age classes) with a development rate fixed to give a median

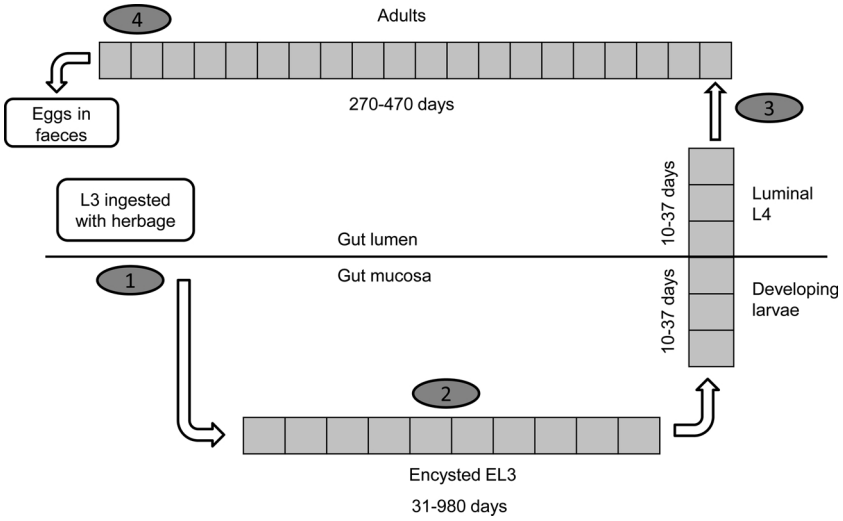

Fig. 1. Schematic representation of the model structure where the boxes represent the arrays in the box-car train, the days indicate the time for 90% of individuals to pass through that stage and the numbers (1–4) correspond to the equations in the manuscript.

life-span for an adult worm of 360 days with 90% of adults dying between 270 and 470 days of age.

2.2.3. Mortality of pre-adult stages

No experimental data exist to describe the mortality of larval stages of cyathostomins within the host, so there are no indications of when, where, or to what degree these stages die off. However, there is evidence that the removal of the established adult burden releases the larval stages from some form of developmental inhibition, allowing a rapid replacement of the adult worm population (Eysker et al., 1989, 1990; Love and Duncan, 1992). The total number of encysted larvae has been reported to far exceed luminal counts, and although this varies by season (Chapman et al., 2003), it is clear that not all larvae make it to the adult stage. Therefore, in the absence of anthelmintic treatments there must be mortality of the larval parasitic stages at some point. In the absence of any data, the model assumes mortality of the luminal L4 stage, invoking a negative feedback from the presence of adult worms to the transition of L4 to the L5 stage. This is implemented as a sigmoid curve with a maximum value of 50% of L4 in the last age class transitioning to L5 each day, declining to almost zero when the adult worm population exceeds 40,000.

$$Trans_{L4} = 0.5 \frac{e^{14-0.0007.WB}}{1 + e^{14-0.0007.WB}} \quad (3)$$

2.2.4. Fecundity and egg output

In general, there is a poor relationship between adult cyathostomin worm burden and faecal strongyle egg count (FEC) in horses (Nielsen et al., 2010). While adult worm burdens remain relatively constant throughout the season, faecal egg counts tend to show a seasonal pattern in temperate climates (Poynter, 1954; Ogbourne, 1975; Chapman et al., 2003), with counts tending to be highest in late spring and early summer and lowest in winter (Ogbourne, 1971; Slocombe et al., 1987). In temperate regions this pattern coincides with a peak in gravid female worms in the spring, where worms appear to be more fecund (Chapman et al., 2003), while a high proportion of female adults are spent during winter (Ogbourne, 1975; Reinemeyer et al., 1986).

As well as a seasonal pattern in egg output there is also an age-related effect. Egg output declines in older horses (Love and Duncan, 1992; Chapman et al., 2003), with horses aged 1–4 years having significantly higher FEC than older age classes (Relf et al., 2013; Lyons et al., 2014). In general, there is a negative correlation between egg count and horse age, and fewer high counts in older horses (Becher et al., 2010). Furthermore, while FECs are over-dispersed in all horses, this tends to be more pronounced in older horses (Relf et al., 2013), suggesting that the greater immune status of adult horses has a suppressive effect on worm fecundity.

To reproduce these patterns in the model, egg production by adult worms was represented by two functions. Firstly, maximum daily egg production was deemed to be a declining exponential function of horse age, from a maximum of 400 eggs/day to 175 eggs/day for each female worm. This maximum number of eggs was then reduced, to about 1/3 of the maximum value, as worms aged beyond about 9 months of age, as;

$$E_i = F_{max} \frac{e^{15-0.52.A_i}}{1 + e^{15-0.52.A_i}} \quad (4)$$

Where, F_{max} = the maximum daily egg production and A_i = the age class of adult worms (1–30).

One study reported the number of eggs present within the uteri of 250 female cyathostomin specimens representing ten different species to range from 31 to 3470 per female, with nine of the species having fewer than 600 eggs present (Kuzmina et al., 2012). While these observations do not allow a direct estimation of cyathostomin fecundity, they do provide some support for the assumptions made in the model.

2.3. Running the model

In order to run and evaluate the performance of this model, it was necessary to simulate an intake of L3 to represent the daily ingestion of larvae with herbage. This was achieved by representing a seasonal pattern of L3 ingestion using a sine curve. This produced an annual cycle of L3 on pasture consistent with seasonal changes and allowed easy manipulation of the numbers of larvae ingested in order to assess their impact on model performance.

A number of studies have measured infection levels on pasture using pasture plucks and the numbers of larvae counted varied enormously among studies, and between seasons within studies, from highs in excess of 100,000 L3/kg herbage (Courtney and Asquith, 1985; Herd and Willardson, 1985; Hutchinson et al., 1989) to counts approaching zero (Reinemeyer and Henton, 1987). Most studies have measured pasture contamination when pastures were grazed by horses in the absence of anthelmintic treatments, which would be expected to have a large impact on the numbers of L3 present (Duncan, 1974; Hutchinson et al., 1989). The intended use of this model is ultimately in the area of anthelmintic resistance management, which means the evaluation of scenarios which involve anthelmintic use, so here we set the larval ingestion rates similar to levels reported in the literature (Reinemeyer

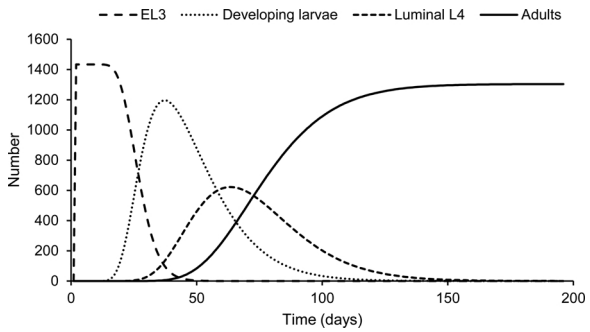

Fig. 2. Number of individuals progressing through different stages within the model when a foal is given a single challenge infection of 10,000 infective larvae.

and Henton, 1987), when anthelmintics were administered. Average daily intakes of 1,000–3,000 were compared with seasonal variations around these means ranging from 100 to 5700 L3 ingested each day.

3. Results

3.1. Assessment of model performance

Initially, the model was run to simulate the development of different parasitic stages following a single challenge infection administered to a previously uninfected and naïve foal. This was done to test whether the model worked without errors and that the duration of different developmental stages was consistent with published literature (Fig. 2). The relationships outlined above produced a median development time from ingested larvae to adult worms of 77 days, with 80% of established larvae becoming adults between 54 and 109 days. The first eggs appear in faeces (at 3 EPG) after about 57 days. The first luminal L4s ($n > 3$) appear on day 25 and the first adult worms ($n > 3$) appear on day 36. In the absence of any anthelmintic treatments, the adult worm burden is relatively constant across a 12-month period, with the exception of an annual decline in numbers which coincides with winter in a temperate region and summer in the tropics (Figs. 3–5). This reflects an annual pattern of decline as the adult worms age, with replacement from the pool of pre-adult larvae.

3.2. Influence of age

The worm burden (all stages) declines with the increasing age of the horse (Fig. 3), which is consistent with the declining establishment of ingested larvae (Eq. (1)). However, the anticipated pattern of a consistently higher ratio of EL3s to adult worms in older compared with younger horses did not always occur. When a 2–4 year old horse and 12–14 year old horse, with an average larval intake of 2000 L3/day were compared, the younger horse had higher numbers of all worm stages, and a higher proportion of EL3 than the older horse (Fig. 3). In contrast, when a horse of 2–4 years was exposed to different levels of intake (an average of 1000, 2000 or 3000 L3/day), both the number of all worm stages and the proportion comprised of EL3 increased directly with the number of L3 ingested (Fig. 4).

3.3. Seasonal and climatic patterns

In addition to the average number of ingested L3s influencing the total worm burden and the relative proportions of the different stages, the seasonal pattern of worm abundance within a year was also influenced by the pattern of larval ingestion. As the amplitude of seasonal increase/decrease in ingested L3s changed, so too did the amplitude of changes in the worm burden, particularly the EL3 stage (Fig. 5). Thus, the anticipated pattern of a higher proportion of EL3 in older horses did occur if the rate of ingestion of L3 with pasture was also higher. Of particular interest was the observation that when the pattern of larval ingestion was changed to reflect either a temperate (summer moist, winter cold) or tropical (summer hot and winter mild) environment, the seasonality of variations in the worm burden also changed (Fig. 5A&B cf C&D). Thus, the behaviour of the model suggests that the rate and seasonality of ingestion of L3 is an important factor which drives both the size and seasonality of worm burdens within the horse.

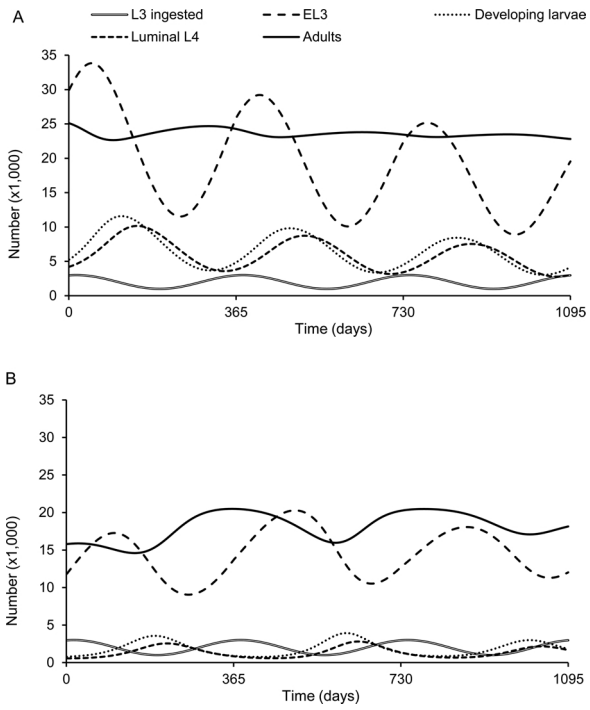

Fig. 3. The seasonal pattern of changes in worm burden in the absence of anthelmintic treatments, under natural (daily) challenge on pasture in a horse which is A) 2 years old or B) 12 years old at the start of the simulation. Note day 1 is about mid-summer. Larval intake varies between 1000 and 3000 L3/day (average = 2000).

4. Discussion

The aim of this study was to describe, in a numerical framework, the dynamics of cyathostomin infection in the horse. While acknowledging that there are limitations to the knowledge on which a model can be based, we propose this model as a working hypothesis, open to challenge, and as the basis for future progress. Ultimately, it is intended to combine this model with its companion, which describes the dynamics of free-living cyathostomins (Leathwick et al., 2015), to build a tool for studying the dynamics of infection and the development of anthelmintic resistance in these parasites. The current exercise was challenging due to a paucity of parameter values, and a considerable lack of knowledge of the key driving variables, a deficiency which undoubtedly reflects the complex biology of the parasites and the difficulties of studying their dynamics in horses. However, the model we have formulated is consistent with current knowledge and reproduces the dynamics of cyathostomin infection as it is currently understood.

For the development of the various parasitic stages, constant rates were used for all stages except the EL3. These were calculated from observations of the times required to reach different developmental stages following first infection in foals. Based on worm counts of infected foals following the ingestion of L3, the first L4 appears in the lumen after 30–74 days (Lyons et al., 2011) and the first gravid females and eggs passed in faeces after about 35–74 days (median 55 days), depending on species (Round, 1969; Reinemeyer et al., 1988; Lyons

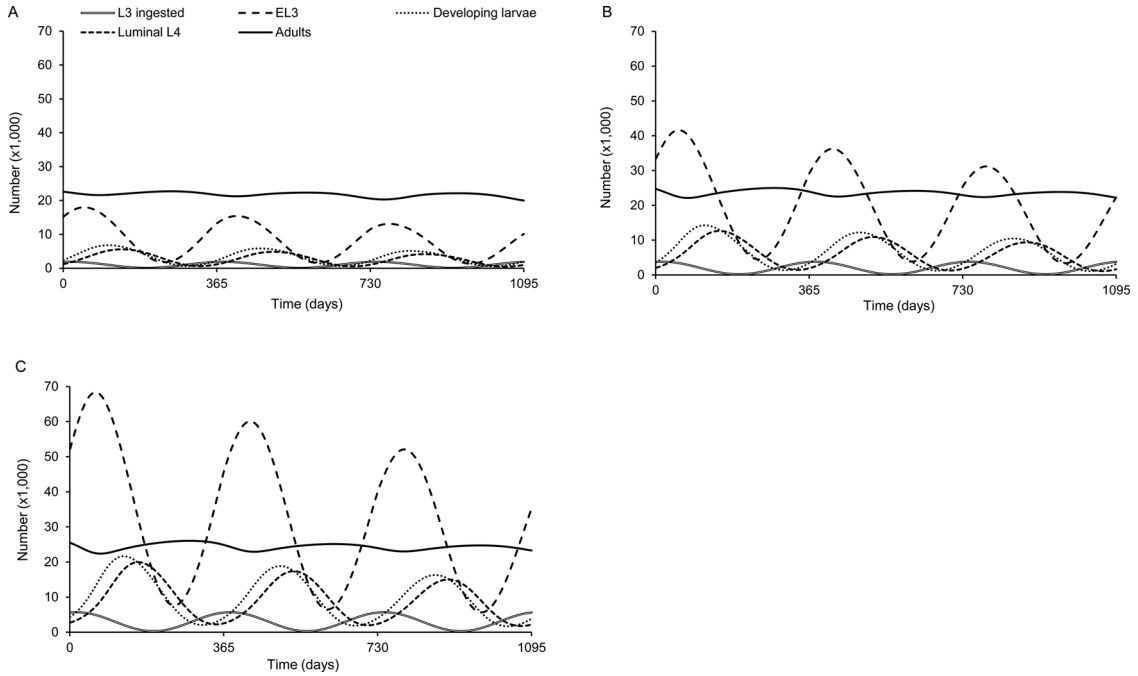

Fig. 4. The effect of different levels of larval ingestion on worm burden in a horse 2 years old at the start of the simulation (*i.e.*, 2–4 y.o.). Average L3 ingested each day = A) 1000, B) 2000 and C) 3000 with a seasonal amplitude of ± 90% of the mean in all cases.

et al., 2011). These values are reproduced in the model, with the first adults present in the lumen after 36 days and the first eggs in faeces after day 57.

In the model, development of the EL3 stage is slowed by the presence of adult worms in the lumen and by the number of L3 ingested with herbage, and this relationship is further modified so that development is slower in older horses. Evidence supporting an effect on EL3 development in response to ingestion of L3 is found in studies which have shown that arrested development was greater in young ponies that were grazed on contaminated pasture and subsequently given a challenge infection compared with equivalent ponies previously kept worm free (Chapman et al., 2002). Other studies have indicated that anthelmintic removal of adult worms from the gut lumen releases earlier developmental stages from some form of inhibition, resulting in a rapid replacement of the adult worm burden (Gibson, 1953; Smith, 1976; Eysker et al., 1989; Love et al., 1999; Mughini Gras et al., 2011). A similar negative feed-back mechanism was suggested in a recent paper describing density-dependent mechanisms of parasite regulation in cyathostomin parasites (Stancampiano and Usai, 2015).

There is also an expectation for lower worm burdens in older horses due to increased levels of anti-parasite immunity (Love and Duncan, 1992; Klei and Chapman, 1999; Collobert-Laugier et al., 2002;

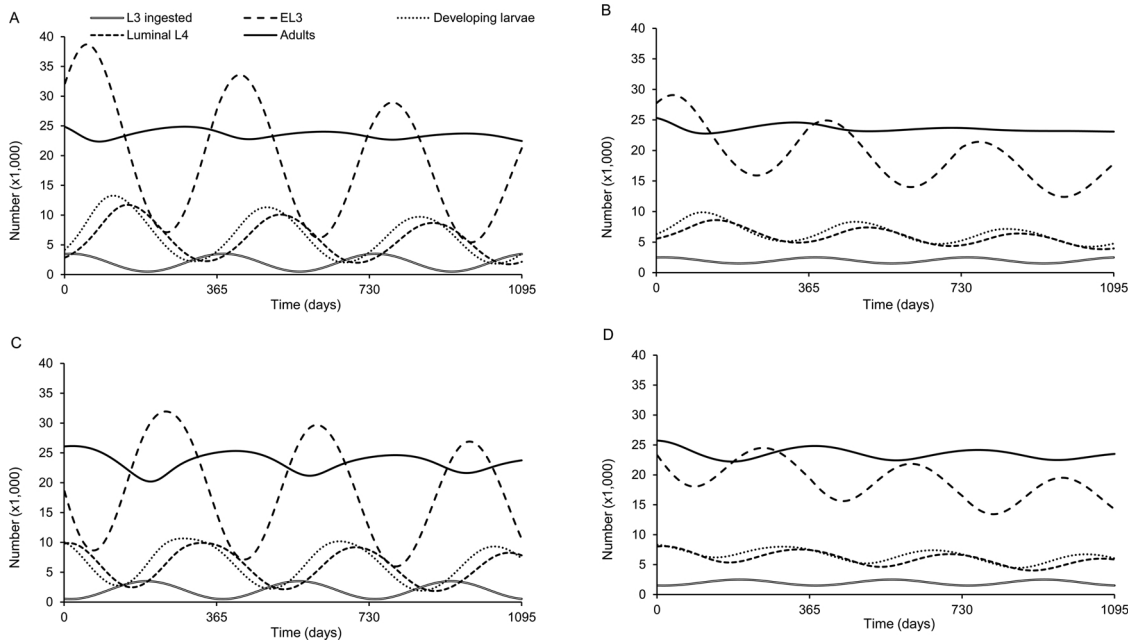

Fig. 5. Effect of varying the amplitude and seasonality of larval ingestion on the magnitude and pattern of worm infection in a horse which is 2 years old at the start of the simulation (*i.e.*, 2–4 y.o.), aiming to represent winter-cold (A&B) and summer-hot dry (C&D) environments, where the average number of L3 ingested daily is 2000 with a seasonal fluctuation of ± 1500 (A&C) or ± 500 (B&D).

Chapman et al., 2002, 2003). However, evidence for this phenomenon is somewhat limited, with few slaughter studies conducted in horses older than about 4–5 years. There is evidence for a reduction in the proportion of EL3 establishing as horses age and become more experienced of worm infection (see Section 2.2.1. above), noting that these two factors cannot easily be separated. Data from Bucknell et al. (1995) and Klei and Chapman (1999) support a general decline in the number of adult worms present as horses age beyond about 2–7 years, but the variation between individual animals within each age group remains high.

In developing this model, we have assumed that adult worms live for approximately one year in the absence of anthelmintic treatments. This is in contrast to previous thinking (Nielsen et al., 2014; Stancampiano and Usai, 2015), however, it does fit with the currently available data. For studies conducted in temperate climates, there was a consistent pattern of worm dynamics which involved a late winter / early spring rise in L4 larvae in the gut lumen, followed soon after by a rise in the proportion of immature adult females. Ogbourne (1975) noted that this maturation of encysted stages in winter/spring coincided with low numbers of L3 on pasture. Gravid females dominate populations through summer and autumn, with spent females being most numerous during winter when adult numbers decline (Ogbourne, 1975; Reinemeyer et al., 1986). This indicates an annual cycle wherein the spring peak in luminal L4 is derived from arrested EL3, which were acquired during previous grazing seasons (summer).

This seasonal pattern indicates that the adult worm burden is not, to any great extent, continuously replaced or supplemented by new adults developing from luminal L4, thereby supporting the concept that the presence of adult worms in the lumen impedes development of the earlier developmental stages. As outlined above, the model includes a negative feedback of adult worm burden on the duration of arrestment at the EL3 stage. In addition, the model also regulates the progression of luminal L4 to adults, which was necessary to stabilise the adult worm burden within a year and allow for the approximately annual life-span (described above). There are currently no data available on the mortality of these parasitic stages, which undoubtedly must exist at one or more stages of the life cycle. Taking a simplistic approach, the model applies no mortality to any of the developing stages until they reach the luminal L4, at which point, in the presence of a blocking influence from adult worms in the lumen, the oldest L4 in the lumen simply die and disappear. Only when the adult worms die at the end of their annual life span, or are removed by anthelmintic treatment, do a proportion of the L4 mature to adult worms. We acknowledge that this is likely to be a crude simplification and that the dynamics and regulation mechanisms are likely more complex. However, not only have no studies attempted to measure the death rates of the different parasitic stages of cyathostomin parasites, but it is difficult to conceive how one would even set about doing this with the diagnostic technologies currently available. Not only is it not possible to sample the same animals repeatedly for an accurate determination of the cyathostomin stages present, but there is invariably a complex of species present which cannot be differentiated as larvae, a very large proportion of arrested EL3s, and large inter-animal variations in worm counts.

Investigation of the performance of the model revealed several interesting features. Firstly, the size of worm populations and the relative abundance of the different stages (*e.g.,* the ratio of EL3 to luminal worms) were strongly influenced by the rate of ingestion of L3 with pasture. With the model maintaining a relatively slow decrease in the establishment rate of ingested L3 as horses age, it follows logically that short-term fluctuations in the numbers of L3 ingested will result in changes in the abundance of larval stages. Because of the blocking effect of adult worms on the final development of luminal L4 (described above), the adult worm burden remains more or less constant over time within a 12-month cycle, and so increasing numbers of EL3 and developing larvae must alter the relative proportions of these when compared to adults. This is in agreement with findings reported in the literature (Chapman et al., 2003).

The numbers of L3 ingested per day by horses is poorly studied or understood, but studies on the availability of infective larvae on pasture demonstrate sizable variations, from close to zero to > 100,000 L3/kg of dry pasture (Courtney and Asquith, 1985; Herd and Willardson, 1985; Reinemeyer and Henton, 1987; Hutchinson et al., 1989). While the grazing habits of horses (Herd and Willardson, 1985) suggest that extrapolating from such extreme numbers to the numbers ingested by a grazing horse is not straightforward, if these numbers are representative of what is occurring on many horse pastures there is clearly ample scope for wide variation in intake of L3. Worm count data from horses, even within similar age classes, typically show high variation in both total numbers and the relative proportions comprised by different stages (Nielsen et al., 2010; Bellaw et al., 2018). Outputs from this model suggest that a proportion of the measured variation in these publications could reflect differences in ingestion rates of L3. Further, the seasonality of L3 availability on pasture was also a significant driver of the rises and falls of the larval stages within the horse. This was perhaps unexpected given the very long periods of arrested development characteristic of this parasite. While seasonal variations in the proportions of EL3 and L4 stages have been documented (Chapman et al., 2003) we are not aware of any attempts to relate these to pasture infectivity.

As outlined above, several studies conducted in temperate climates have shown a consistent pattern of worm dynamics. This pattern involves adult worms dying off over-winter, to be replaced by new adults derived from the pre-adult stages, giving rise to a new population of highly fecund adult worms over spring-summer (Ogbourne, 1975; Reinemeyer et al., 1986). However, variations on this pattern are evident. In environments which experience hot, dry summers and mild, moist winters, a similar cycle has been observed, but with a different seasonality. A study in Louisiana (Chapman et al., 2003) found that EL3 were at their highest levels in summer and lowest in autumn (fall), whereas adults made up the bulk of the worm burden in autumn and were at their lowest in summer. Furthermore, the adults present in fall also appeared to be more fecund (Chapman et al., 2003). This indicates a pattern wherein EL3 emerge as L4 following a decline in adult numbers over summer to establish a new population of adults in the autumn. The adults persist through winter and spring before declining again over the next summer.

Hence, in the two different environments, an annual cycle appears to have developed which ensures survival (*via* arrested EL3) over the period of adverse environmental conditions, whether that be winter or summer, when survival of free-living stages is at its lowest. Interestingly, the model reproduces these two patterns based solely on changing the seasonal pattern of ingestion of L3 on pasture. Accordingly, if the numbers of L3 on pasture are highest in summer and lowest in winter, the model output reflects that seen in temperate environments (Fig. 5A and B), whereas if the L3 on pasture peak in winter and are lowest in summer, the pattern is typical of tropical or subtropical environments (Fig. 5C and D).

While this may not be surprising given the structure of the model, it does suggest that worm dynamics in different regions of the world can be reproduced without any requirement for additional variables. It has been suggested that arrestment of L3 may be related to season, *i.e.*, some form of preconditioning of the L3 on pasture (Reinemeyer, 1999; Schánková et al., 2014), and similar findings have been reported for ruminant trichostrongylid parasites (Smeal and Donald, 1982). However, the model reproduces the seasonal pattern of dynamics within the host without accounting for this variable. Therefore, while 'conditioning' of L3, which predisposes them to arrest, cannot be ruled out, the model suggests that such external variables are not required to reproduce the appropriate pattern of worm dynamics. This is an important outcome given that it is intended to combine this model with an earlier one describing the dynamics of cyathostomins on pasture (Leathwick et al., 2015), and its applicability to different climate zones

will be important.

It is worth noting that larval cyathostominosis, which is a disease complex associated with mass emergence of encysted larvae (Love et al., 1999), appears to be seasonal in occurrence. A British investigation reported cases occurring most often between November and March and to be associated with recent anthelmintic treatment (Reid et al., 1995), while a study conducted in Ontario, Canada found most cases occurring between October and December (Peregrine et al., 2006). While the model reported herein was not developed to predict disease risk, it does suggest that climates offering large seasonal variations in the number of L3s ingested also leads to larger encysted burdens (Fig. 5). Both Ontario, Canada and the United Kingdom offer climates with large winter-to-summer differences in parasite transmission, which could explain why a majority of cases have been reported from these countries. In contrast, equine veterinarians in the state of Kentucky virtually never encounter larval cyathostominosis, despite a high concentration of young horses (Nielsen, M.K., personal observation).

In summary, we have developed the first computer model simulating all parasitic stages of equine cyathostomin parasites. The model outputs are in line with data reported in the published domain, and appear to provide meaningful insights into population regulation. However, the model building exercise has also identified gaps in our understanding of these parasites' biology and dynamics. Future work will aim at combining this model with an already published model describing the development and survival of the free-living cyathostomin stages (Leathwick et al., 2015), and will then proceed with simulating development of anthelmintic resistance under various anthelmintic treatment regimens.

Conflict of interest

We the authors declare no conflict of interest in relation to this publication

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